Nanoscopy

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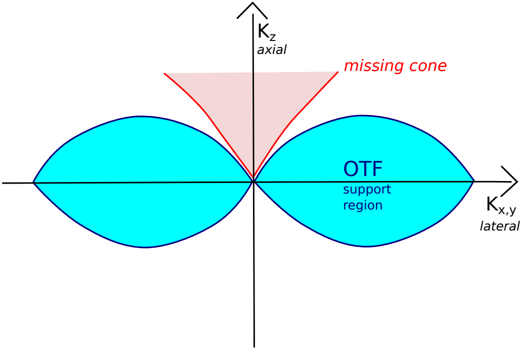
The missing cone problem

- **Lateral resolution in focus:** Easy, Abbe limit.
- **Axial resolution depends on lateral frequency** (and vice versa).
- Counter-intuitive? Think about (de)focusing a very fine or very coarse structure.



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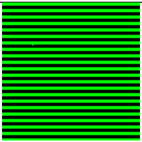
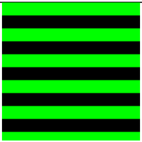
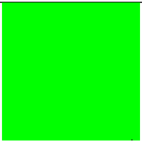
Missing Cone: Visualization

	Fine	Medium	Coarse / const.
Sample (w/o diff. limit)			

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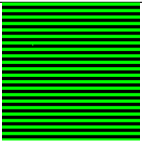
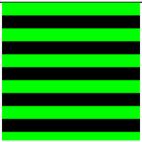
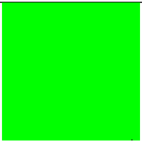
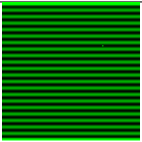
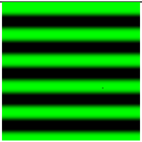
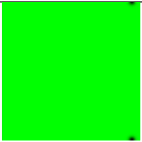
Sample (w/o diff. limit)			
Sample in focus			

Missing Cone: Visualization

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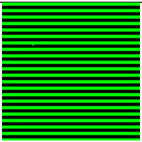
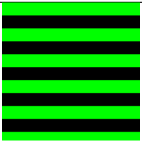
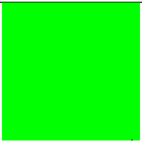
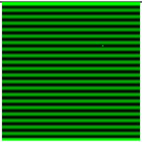
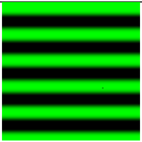
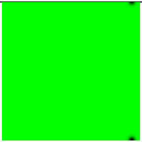
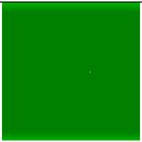
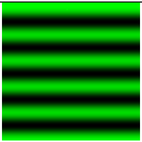
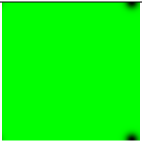
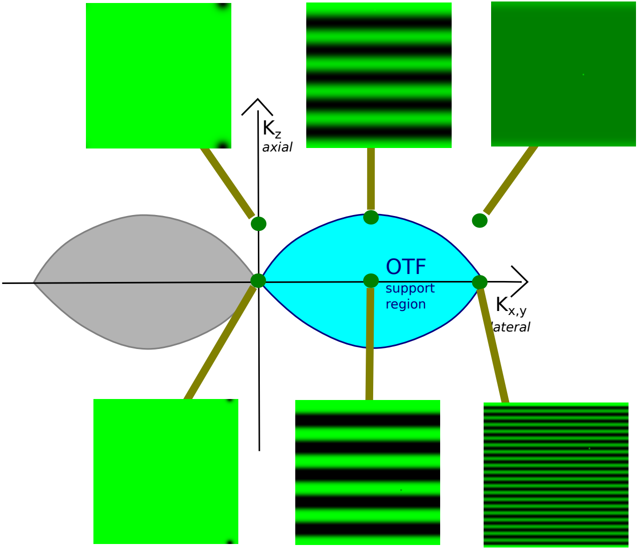
Sample (w/o diff. limit)			
Sample in focus			
Sample defocus			

Missing Cone: The full picture



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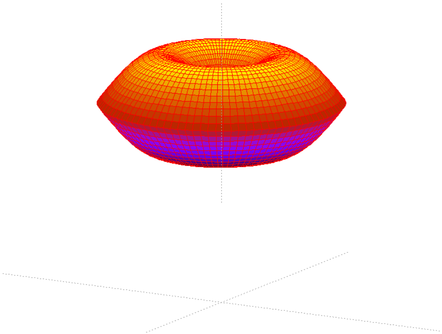
Widefield 3d OTF - missing cone



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Missing cone problem affects all wide-field measurements.
It has to be circumvented for successful structured illumination microscopy.

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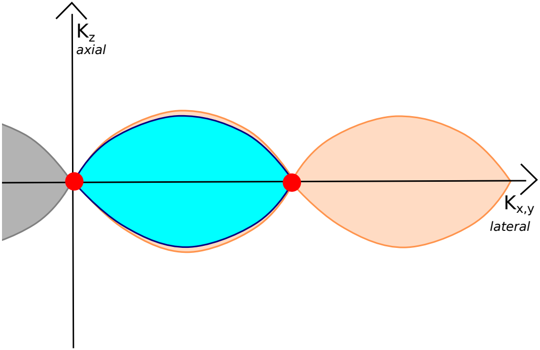
2D SIM - shifted copy of the OTF

- k-space position of illumination pattern: copy of the OTF
- At limit of support: Doubles resolution
- Now missing three cones!



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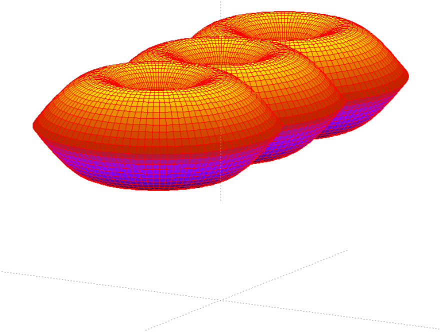
SIM at max. lateral resolution improvement - 3 missing cones



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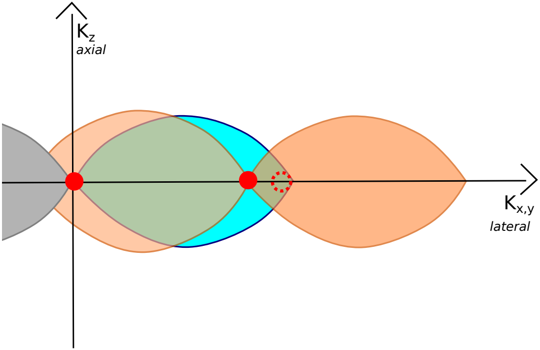
2D SIM - compromise resolution / sectioning

- SIM with one pattern: Trade-off between optical sectioning (axial resolution) and lateral improvement
- With $1.6\times - 1.8\times$: Easy to build, easy to analyse, fast (9 images), better than confocal
- Missing cone filled



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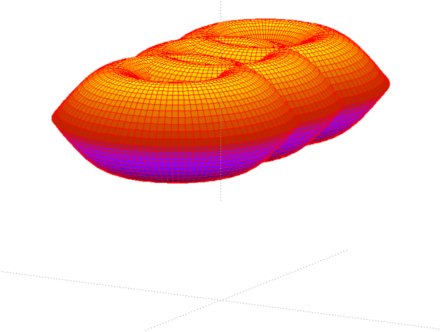
SIM at intermediate lateral resolution improvement - cones filled



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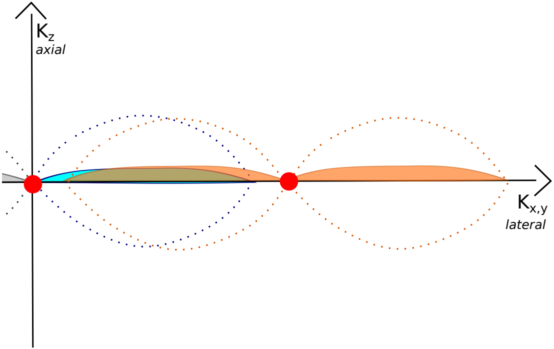
TIRF 2D SIM

- TIRF: A bit more than 2×, caused by the TIRF angle.
- Pattern beyond OTF: hard to estimate parameters correctly
- Fast, only 9 images
- All wide-field drawbacks of TIRF



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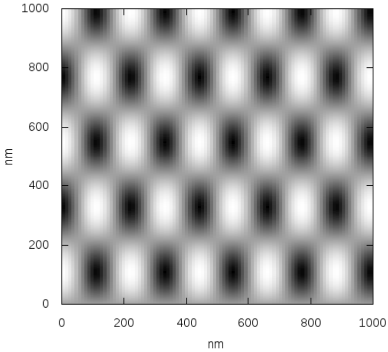
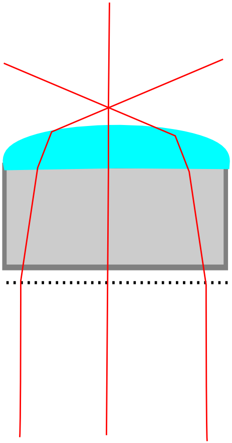
3D SIM by Gustafsson: OMX, Elysa, . . .

- Pattern generation by 3 interfering beams: Center, left/right corner.
- Generates two superimposed patterns: $I = \sum_{m=0}^{2} I_0 \cdot exp(im\phi)$





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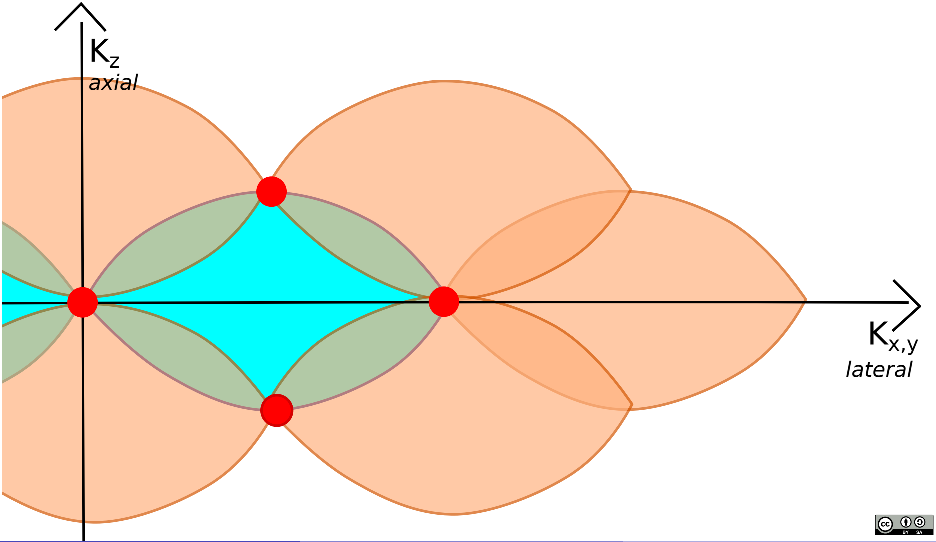
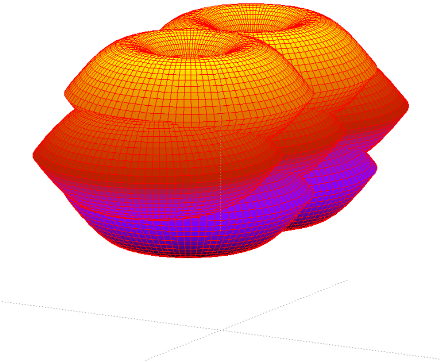
OTF extension lateral / axial

- Complex modulation pattern fills axial and lateral Fourier space
- Be careful how to determine / define axial resolution



Full 3d SIM with 2 modulation frequencies and axial offset



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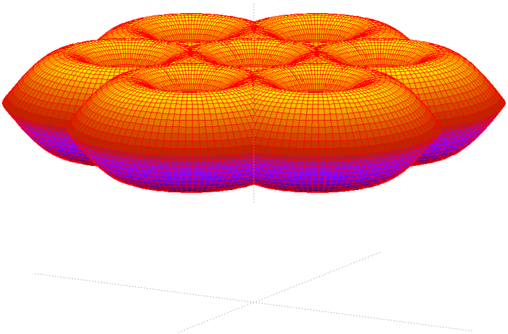
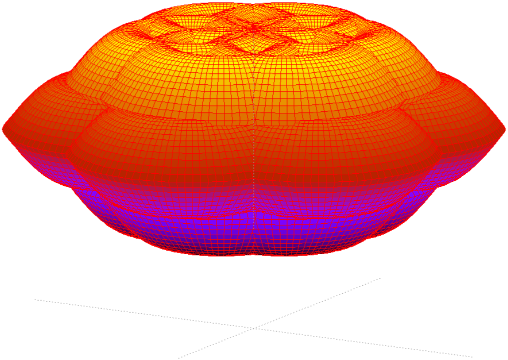
Maximum lateral improvement, all angles



Full 3d SIM, all angles



3D reconstruction

- **"real" 2D SIM**
  Only data in focus: Ideal OTF with estimated cut-off works o.k.
- **full 3D SIM**
  Correct (axial) OTF is critical
- **2D reconstruction of 3-beam data**
  - Axial OTF projection. Less precision than 3D, still more complex than 2D.
  - OTF attenuation to obtain section: Otherwise, background will ruin reconstruction.

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Deconvolution-based SIM

Image formation in SIM

$$M_l(x,y) = \int_{S_z} \mathrm{PSF}(x,y,z) * \left(I_l(x,y,z) \cdot S(x,y,z)\right) \mathrm{d}z \qquad (1)$$

$$I(x,y,z) = \mathrm{PSF}_{\mathrm{ex.}}(x,y,z) * I'(x,y,z) \qquad (2)$$

- Interested in: **Sample response** (fluorescence density) $S(x,y,z)$.
- Limited by: PSF, i.e. Abbe limit lateral, background axial
- All SIM approaches:
  - Obtain $S(x,y,z)$
  - by combining multiple measurements M_l
  - for different illuminations $I_l(x,y,z)$
- **Keep in mind**: Illumination also limited by $\mathrm{PSF}_{\mathrm{ex.}}$.

Deconvolution-based SIM: Principle

$$M_l(x,y) = \int_{S_z} \mathrm{PSF}(x,y,z) * \left(I_l(x,y,z) \cdot S(x,y,z)\right) \mathrm{d}z \tag{3}$$

Deconvolution-based approaches: Treat $M = [M_0, \ldots, M_n]$ as one vector, solve as an equation system.

- \+ Works for arbitrary light modulations $I_l(x,y,z)$
- \+ Allows for corrections, i.e. aberrations
- – System is not linear, thus complex solvers.
  i.e. non-linear iterative, MEM, MLE:
  "unphysical" regularization, computationally expensive

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Deconvolution-based SIM: Methods under development

$$M_l(x,y) = \int_{S_z} \mathrm{PSF}(x,y,z) * \left(I_l(x,y,z) \cdot S(x,y,z)\right) \mathrm{d}z \tag{4}$$

- **Fully blind SIM**:
  Minimal prior knowledge of $I_l(x,y,z)$, i.e. $\sum_l I_l(x,y,z) = \text{const.}$
  Proof-of-principle paper: *Unknown Speckle Illumination*
- **Partially blind SIM**:
  $I_l(x,y,z)$ mostly known, but allowed to vary within limits.
  Useful for aberration correction. *FOM2015: A. Jost, R. Heintzmann*
- **Standard SIM, abitraty illumination**:
  $I_l(x,y,z)$ known and fixed, but no need for "nice" Fourier space decomposition
- **Pobable likelihood estimation**:
  Automatic reconstruction procedure for physically plausible results.

Outlook

Next weeks: Localization microscopy

SIM image processing

Hands-on image processing by Wolfgang